

Research Article

Effect of the Fruits Type and Conservation Time in the Germination Capacity of *Jacaranda (Jacaranda mimosifolia D. DON)* Seeds Collected in Alentejo, South Portugal

Orlanda Póvoa*

Department of Agriculture and Natural Resources, Polytechnic Institute of Portalegre, Portugal

*Corresponding author

Orlanda Póvoa, Department of Agriculture and Natural Resources, Polytechnic Institute of Portalegre, Portugal, Tel: 351-268-628-528; Fax: 351-268-628-529; Email: opovoa@esaelvas.pt

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Abstract

Jacaranda is a deciduous tree from South America, used as an ornamental tree due to its abundant flowering, usually found in South Portugal. The tree also has forest interest.

Fruits were harvested in 2010, 2013, 2015 and 2017 in the city of Elvas. From the samples collected in 2013 and 2017, two fruit subsamples were obtained: a) dark brown fruits, whose maturation was reached in the previous year and b) light brown fruits, whose maturation was reached in the harvest year. In 2015, 2 subsamples were also collected: a) dark brown fruits and a mixture of all fruits types. The samples were threshed, labeled and conditioned in a refrigerator (ca. 5°C). The germination assays were carried out at 20°C with 12 h of photo phase. Final observations were obtained after 21 days.

The germination results ranged from 11.3% (dark brown, old fruits) to 93.5% (light brown, new fruits). The best results, without significant statistical difference between samples, ranged from 70 to 93.5%, indicating that the seeds of this species harvested in Alentejo (Elvas) have high germination capacity. In the fruit harvesting operations, it is important to separate the fruits, because in the 3 tested samples of seeds harvested in different years, the seeds obtained from light brown, new fruits, had higher germination rates (9 to 35% more), with significant statistical difference from dark brown, old fruits. The seeds harvested in 2010 maintained their germination capacity (71%) when stored in a refrigerator (ca. 5°C) for 7 years.

INTRODUCTION

Jacaranda mimosifolia D. Don (*Bignoniaceae*) is a deciduous tree of 12-15 m of height from South America, used as an ornamental tree due to its abundant flowering, usually found in South Portugal. It also has forest production potential. The plant is also used medicinally, being used for its antibacterial action against *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus* in Pakistan [1].

In south of Portugal, Alentejo region, this tree blooms in May and June [2], after which the capsules grow and remain green until August, after which they acquire an increasingly intense brown color. The seed maturation occurs the following spring being evidenced by the opening of the capsules; the capsules remain in the tree for a long time, acquiring a dark brown color

due to the development of fungi. In spring, several types of capsules may be observed in the trees; recently matured, light brown, at the beginning of the dehiscence; dark brown, open capsules with varying amounts of retained seed and; very dark capsules, without remaining seeds.

The species propagates by seed. In the works of Sokolowski et al. [3], the species germinates from 10°C to 40°C, with optimum at 25°C (with values close to 60%), although without statistical differences between 20°C and 30°C. In the works of Macielet al. [4], the best germination results were also obtained with a temperature of 25°C (54% germination), although without statistical difference from the alternate temperature of 20/30°C. Myajima et al. [5], obtained 80.3% germination in vitro with seeds harvested 5 months after fertilization.

From the practical point of view, it is more pragmatic for tree

seed collectors not to triage the fruits, which can compromise the quality of seed lots, which justified this study.

The goals of this study were to evaluate the germination capacity of the seeds of the species collected in Alentejo region, taking into account the diversity of fruits that usually remain in the trees throughout the year, as well as their long term conservation in a laboratory refrigerated environment. Evaluating the influence of the collection site, namely the influence of the proximity to the Atlantic Ocean was another goal.

MATERIALS AND METHODS

The fruits were harvested in 2010, 2013, 2015 and 2017 (Table 1) from 3 different gardens, all with watering systems; Seixal city as Atlantic Ocean proximity and Elva's city as continental climatic conditions, being located inland Portugal. The samples were collected from groups of trees, except for Elvas (ESAE) sample with an individual tree.

From the two samples collected in 2013 and 2017, fruit subsamples were obtained: a) dark brown fruits, whose maturation was reached in the previous year and b) light brown fruits, whose maturation was reached in the harvest year (Figure 1). In the year of 2015 also 2 subsamples were made: a) brown-dark fruits, whose maturation was reached in the previous year and a sub-sample with mixture of all types of fruits.

The samples were threshed, identified and conditioned in a refrigerator (ca. 5°C) in the Laboratory of Plant Biology of the Elvas Agrarian School of Polytechnic Institute of Portalegre.

Germination tests were performed using a methodology similar to that used by Póvoa et al., and Póvoa et al [6,7]. The various germination assays were performed at 20°C with 12 h of photophase in a growth chamber. Four replicates of 100 seeds were used for each sample; the seeds were placed on 9 cm Petri dishes in top of filter paper. No seed sterilization method was used. The observations of germinated seeds were made 2 times

per week. Final observations were obtained after 21 days.

The weight of 1000 seeds was estimated from weighing 4 samples of 100 seeds on a precision laboratory balance.

Data were recorded in tables in Excel software and the means, standard deviation and coefficient of variation were calculated. The results were submitted to an ANOVA (variance analysis), using the Tukey's Honestly Significant Difference Test (Tukey's test), using 0.05 level of significance, for comparison and separation of means, both performed with the STATISTICA version 8.0 program [8].

RESULTS AND DISCUSSION

Although the visual appearance of the seed samples are similar to naked eye, seed germination was markedly different. The best germination result was 93.5% (fresh fruits) (Table 2,3). The new fruits have seeds that germinate better than the old fruits, with highly significant statistical difference; the 'mixed' fruits, without separation of old and new fruit subsamples, have seeds that germinate better than the old fruits, with significant statistical difference.

The seeds harvested in Elvas in April of 2015 germinated worse than the others, with significant statistical difference; most likely due to the fact that this sample contains a mixture of all the fruits present in the trees, with predominance of old fruits. No differences were found in the germination capacity of seeds harvested in 2015 at Seixal (coast of Portugal) when compared to Elvas (Portugal inland). The productive conditions from mother plants, namely variation on local climatic conditions during different collection years, influence seed germination and seed dormancy [9]. Further collection sites should be tested in future to reinforce these results.

It is concluded that the germination results of this species depend on the sample, and can vary from 11.3% (worse result, with old fruits) to 93.5% (best result, with new fruits).

Table 1: Samples of *Jacaranda mimosifolia* tested seeds.

Trial	Trial date	Harvest date	Sample description
2013	May 2013	30 April 2013	Elvas (city center, group of trees), light brown new fruits
			Elvas (city center; group of trees), dark-brown old fruits
			Seixal (group of trees); light brown new fruits
2015	April 2015	15 April 2015	Elvas (ESAE, individual tree), light brown new fruits
			Elvas (Praça 25 Abril, group of trees), dark-brown old fruits
			Elvas (Praça 25 Abril, group of trees), mixture of fruits
2016	December 2016	05 April 2010	Elvas (Praça 25 Abril, group of trees), harvest in 2010; refrigerator storage (ca 5.°C); light brown new fruits
		15 April 2015	Elvas (ESAE, individual tree), light brown new fruits
		9 June 2017	Elvas (ESAE, individual tree), dark-brown old fruits
2017	June 2017	5 April 2010	Elvas (Praça 25 Abril, group of trees), harvest in 2010; refrigerator storage (ca 5.°C); light brown new fruits
		15 April 2015	Elvas (Praça 25 Abril, group of trees), harvest in 2015, refrigerator storage (ca 5.°C); dark-brown old fruits
		15 April 2015	Elvas (Praça 25 Abril, group of trees), harvest in 2015, refrigerator storage (ca 5.°C); mixture of fruits
			Elvas (ESAE, individual tree), harvest in 2015, refrigerator storage (ca 5.°C); light brown new fruits



Figure 1 Old fruit (left), new fruit and its seeds (center) and; germinated seed (right) from *Jacaranda mimosifolia*.

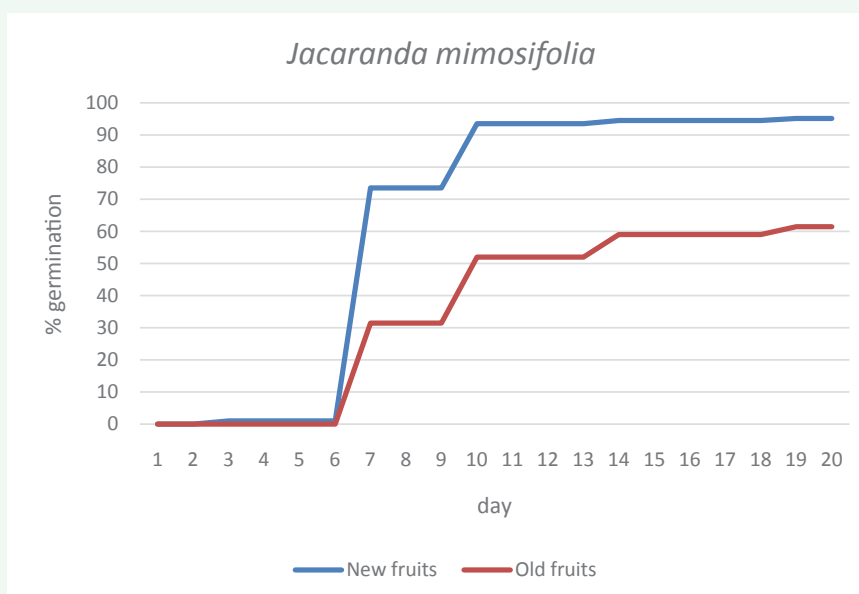


Figure 2 Accumulated germination of *Jacaranda mimosifolia* in the 2013 trial. Average of 4 replicates of 100 seeds.

Germination begins 3 days after the beginning of the trials, with the majority of seeds germinating in the first 7 days (Figure 2).

In the works of Sokolowski et al., Maciel et al. [3,4], (20%), the best germination results (54%) were obtained with the temperature of 25°C, although without statistical difference of other temperatures (20°C, 30°C and 20/30°C). The results obtained in the present work (the best result was 93%) exceed these bibliographic germination. Myajima et al. [5], obtained 80.3% germination in vitro with seeds harvested 5 months after fertilization. The best results of the present study exceed the best results of all the cited bibliographic references.

In the fruit harvesting operations, it is important to separate the fruits, because in the 3 samples of tested seeds harvested in different years, the seeds obtained from new fruits obtained superior germination (35% more), with a high significant

statistical difference from the old fruits. The partial separation of fruits, eliminating the older fruits, also resulted in germination gain (15.3%), as evidenced by the results of germination of the sample harvested in Elvas city in 2015.

It has been established long ago that fungi deteriorate seed quality during storage [10,11] fungus also contribute to seed deterioration after seed maturation, especially on years with long periods of rain. However, some fungus may reduce seed coat hardness and increase germination [12]. Insects may also interact with fungus, contributing to seed deterioration when the harvest is delayed [13].

Our results put in evidence that *Jacaranda mimosifolia* seed quality decreases when harvest is delayed, thus old fruits should be removed during seed collection.

The seeds harvested in April 2010 and stored in a refrigerated environment maintained the germination capacity until 2017

Table 2: Results of variance analysis (ANOVA) of the germination of the 14 seed samples of *Jacaranda mimosifolia*.

Trial	Harvest site	Harvest date	Fruits type	Average Germination (%)	*	Standard deviation	Variation coefficient (%)
2013	Elvas	April 2013	new	93,5	a	7,55	8,1
2017	ESAE	June 2017	new	90,7	a	5,03	5,6
2016	ESAE	April 2015	new	82,7	ab	9,45	11,4
2015	ESAE	April 2015	new	72,7	ab	1,15	1,6
2017	Elvas	April 2010	new	71,3	ab	10,07	14,1
2017	ESAE	April 2015	new	70,9	ab	7,83	11,0
2015	Elvas	April 2010	new	70,0	ab	15,62	22,3
2013	Elvas	April 2013	old	59,0	b	8,87	15,0
2015	Seixal	April 2015	new	56,7	b	12,70	22,4
2017	ESAE	June 2017	old	55,6	b	11,98	21,6
2015	Elvas	April 2015	mixture	26,7	c	8,08	30,3
2017	Elvas	April 2015	mixture	20,7	c	8,33	40,3
2017	Elvas	April 2015	old	12,7	c	10,07	79,5
2015	Elvas	April 2015	old	11,3	c	3,06	27,0
Average				56,7			
Standard deviation				28,1			
Variation coefficient (%)				49,5			
Significance (<i>p-Value</i>)				p≤0.05			

Abbreviations: Values with the same letter in the column do not differ significantly.

Table 3: Results of variance analysis (ANOVA) of the germination of *Jacaranda mimosifolia* seeds for fruit type factors, harvest site and harvest date.

Factor	Average Germination (%)*	*	Significance (<i>p-Value</i>)
Fruits Type			
New	65,6	a	
Mixture	23,7	b	
Old	34,6	c	p≤0.001
Harvest site			
ESAE	74,5	a	
Seixal	56,7	b	
Elvas	45,6	b	p≤0.05
Harvest date			
Abr13	76,3	a	
Jun17	73,1	a	
Abr10	70,7	a	
Abr15	44,3	b	p≤0.001

Abbreviations: *Values with the same letter in the column do not differ significantly.

Table 4: Results of variance analysis (ANOVA) of the weight *Jacaranda mimosifolia* seeds for fruit type factors, harvest site and harvest date.

Harvest site	Fruits type	Harvest date	100 seedsweight (g)	*	Standard deviation	Variation coefficient (%)	Seeds/kg (number)
Elvas	new	April 2010	0,72	b	0,02	5,64	138312,6
Elvas	mixture	April 2015	0,68	b	0,01	1,57	148038,5
Elvas	new	April 2015	0,92	a	0,04	8,40	108991,8
ESAE	new	June 2017	0,95	a	0,01	1,86	105596,6
Average			0,85				120875,6
Standard deviation			0,15				23584,9
Variation coefficient (%)			17,59				19,5
Significance (<i>p-Value</i>)			p≤0.0001				

Abbreviations: *Values with the same letter in the column do not differ significantly.

(71.3%), without statistical difference from the seeds harvested in 2017 (90.7%).

It is possible to maintain the germination capacity of the seeds of this species in a refrigerated environment (about 5°C) for at least 7 years.

The weight of the seeds (Table 4) is variable with the sample, on average 1000 seeds weighs 8.47 g; the seeds of the 2010 harvest and the seeds of the aged fruits of 2015 were lighter, differing statistically from the remaining 2 samples.

CONCLUSION

The results of germination ranged from 11.3% (dark brown, old fruits) to 93.5% (light brown new fruits). The best results, without significant statistical difference between samples, ranged from 70 to 93.5%, indicating that the seeds of this species harvested in Alentejo (Elvas) have high germination capacity, surpassing the values published in the consulted bibliography.

No differences were found in the germination capacity of seeds harvested in Seixal (coast of Portugal) when compared to ESAE - Elvas (Portugal inland).

In the fruit harvesting operations, it is important to separate the fruits, because in the 3 samples of tested seeds harvested in different years, the seeds obtained from new fruits obtained superior germination (35% more), with a highly significant statistical difference in relation to the old fruits.

The weight of the seeds is variable with the sample, on average 1000 seeds weighs 8.47 g; 1 kg has on average 12087.6 seeds.

The seeds harvested in 2010 maintained their germination capacity (71%) when stored in a refrigerator (ca. 5°C) for 7 years.

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